Mast cells and glycosaminoglycans in oral mucosa in Behcet's disease

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Summary

***Objective*. Mast cells (MC) and glycosaminoglycans (GAG) in various zones of oral mucosa are investigated during remission and aggravation of Behcet disease (BD) in 17 patients (15 men and 2 women; average frequency of aggravations in a year - 4.5).**
***Methods*. Bioptats (biopsy material) of various zones of oral mucosa are investigated histochemically. The MC are identified by 0.05 percent thionine solution according to Hassanov's prescription, as well as by GAG-PAS-reaction under the control of amylase, and by fast blue and strong garnet CBS [1]. The statistical correlation between frequency of aggravations of BD and histochemical quantitative indices of the MC and GAG is studied.**
***Results*. During remission of BD the MC are distributed chaotically on Lamina propriae of oral mucosa, they are mainly under epithelium and throw out contents of granules weakly (degranulation index - 3.0-5.0%). In BD aggravations the total number of MC remains without obvious changes, they come to light near small vessels. Secretion of histamine-like substances sharply increases. Redistribution and increase of secretory activity of the MC are evident in the zone of aphthae and beyond their perimeter. During BD remission the GAG are histochemically verified in lamina propriae of oral mucosa. The proportion of neutral and sour GAG during these periods: 1.7/1.3. During aggravation of BD, the histochemical content of GAG markedly rises over the whole oral mucosa and their quantitative redistribution occurs. The proportion of neutral and sour GAG in the zones of aphthae and periaphthae sites is 1.1/1.9.**
***Conclusion*. Aggravation of BD is directly proportional with activation of the MC and increase of histochemical content of sour GAG in zones of aphthae and periaphtous sites of oral mucosa.**

***Keywords*: aphthous stomatitis, Behcet disease, mast cells, glycosaminoglycans.**

Substantiation of the subject of research

Behçet Disease (BD) is a chronic inflammatory disease with a multisystemic affection of unknown etiology [1].

Basic manifestations of the disease are represented by a triad of clinical symptoms: recurrent aphthous stomatitis, necrotically ulcerated changes in mucous membrane of genitals, inflammatory affections of eyes [2,3,1]. Over the last 50 years new localizations of manifestations have been revealed – joints, gastrointestinal tract, cardiovascular system and nervous system [4].

According to modern classification of rheumatic diseases, BD is considered to belong to vasculitis [5].

Critical analysis of the literature has shown that an important pathogenetic link in BD is vessels microcirculation stream and tissue elements connected with them. The

quantities, total area of the gap of functioning vessels as well as permeability of their walls are regulated by a multitude of general and local mechanisms. A special importance among the latter is attached to the so-called “mast cells”.

Mast cells or labrocyte, (granulocytus basophilis textus) were for the first time described in 1887 by P. Ehrlich. Characteristic of mast cells is the presence of large specific granules (0.3-0.7 microns in diameter), stainable metachromatically by thiazine dyes in their cytoplasm. They are an indispensable component of the connective tissue, are observed in all cases when even small layers of it are met. They synthesize, accumulate and secrete biogenic amines: histamine, serotonin, dopamine. The perivascular arrangement is predominantly characteristic for these cells [6,7,8].

The importance of mast cells in regulation of the microcirculation stream of oral mucous membrane in BD remains poorly studied. Analyses by publications and related research are few, they are performed on the basis of meager actual information and their results are contradictory.

The second tissular components of vasoregulating importance are acid glycosaminoglycans (GAG) of intermediate substance of connective tissue of oral mucous membrane. As it is known, acid glycosaminoglycans are divided in two groups – sulphated and nonsulphated [7,8].

Analysis of the publications has demonstrated that the condition of acid (sulphated and nonsulphated) GAG in connective tissue of oral mucous membrane in aphthous stomatitis in patients with BD is practically not explored.

Object of the research

To carry out clinical and morphological study of oral mucous membrane during aggravation and remission of BD with special emphasis on the importance of immune status, microcirculatory stream, mast cells and acid GAG of oral mucous membrane.

Material and method

17 patients with BD were examined: 15 men, 2 women; their average age was 29.9, and the average duration of the disease was 6.1 years. The condition of their mouth cavity was assessed by a dentist. In all cases the process was traced both during aggravation and remission periods. Duration of observations lasted from 1 to 3 years.

In parallel, the oral mucous membrane of 20 patients with chronic recurrent aphthous stomatitis of not-Behcet genesis was studied. These patients served as control group. It comprised 12 men and 8 women, whose average age was 22.4 and the average duration of aphthous stomatitis was 2.7 years.

In parallel with general stomatological analysis, histological, histochemical and immunohistochemical investigations were also carried out. For these purposes small bioptats (1-3 mm) of oral mucous membrane were taken from aphtae, periaphthous zones and remote sites. Part of the bioptats were processed histopathologically, and others were studied by histochemical and immunohistochemical means, after freezing on solid carbonic acid. Microtomic and cryomicrotomic sections were obligatorily stained with hematoxylin-eosin and picrofuchsin. Mast cells were assessed by 0.5% buffer solution of thionine with pH equaling to 5.5 according to I.A. Hassanov’s method (1997) [9]. With the help of the same dye acid non-sulphated GAG were also studied. Check samples were also taken during the mentioned histochemical reactions without fail. The sulphated acid GAG were determined through PAS-reaction under the check of amylase as well as of fast blue and strong garnet CBS.

Alongside with light-microscopy research, electron-microscopy analysis of

samples of oral mucous membrane of a limited group of patients (4 from the control group, 6 from the main one) by JEM-100S microscope was made (JEOL, Japan).

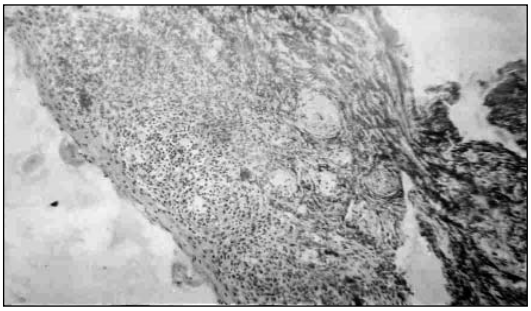
Histopathological analysis of the oral mucous membrane was made. Qualitative and quantitative analyses were performed histochemically. The distribution density of mast cells (the number of cells/mm^2), specific weight of degranulating cells as well as visual quantitative contents of acid GAG were determined by 4 mark scale (1.0 – absence, 4.0 – maximum content of substance). The obtained figures were processed by statistical methods for parametrical and not parametrical criteria with calculation of correlation coefficient (r), and Pearson criterion (χ^2). A statistical correlation analysis of dependence between frequency of aggravations of BD and histochemical quantitative indices of mast cells and GAG was performed.

Results and discussion

At histopathological analysis of oral mucous membrane of patients from the control group with chronic recurrent aphthous stomatitis of not-Behcet genesis, during aggravation, erosive mucositis with angio-granulation in lamina propriae and defect of epithelial cover in aphthae zones are observed. In periaphthous sites, swelling of lamina propriae, deformation of its microcirculatory stream and minor perivascular lymphoid infiltration under epithelium are present. Remote sites are without features. (*Figure 1*)

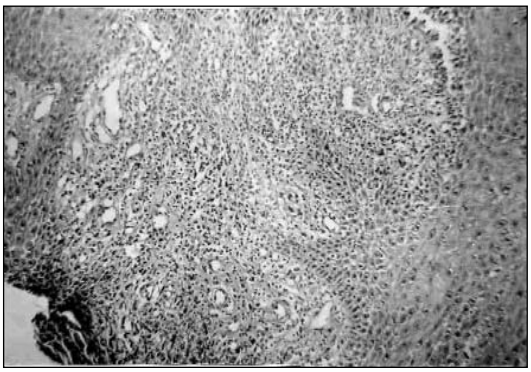
Destruction of epithelial lamina with fibrinous detritus, "non-specific" microvasculitis against the background of oedema of lamina propriae of oral mucous membrane are met in patients with BD in the zone of aphthae during aggravation. Inflammatory infiltrations contain approximately equal quantities of polymorphonuclear leucocytes, lymphocytes and plasmocytes.

Figure 1. Histological condition of oral mucous membrane during aggravation, in patient with chronic recurrent aphthous stomatitis (control group). The bottom of aphthae. Patient A., 29 years old. Thionine, 300 x



Perivasal infiltrations of similar cellular composition but of smaller sizes and the degrees of expression are also found in the adjoining zones of oral mucous membrane (*Figure 2*).

Figure 2. Histological aspects during aggravation period in patient with aphthous stomatitis in BD. Diffusive microvasculitis of lamina propriae. Compound cell composition of inflammatory infiltration. Periaphthous zone. Patient V., 29 y.o. Thionine, 300 x



Such aphthae are not observed in the patients from both control and main clinical groups during remission period of aphthous stomatitis; a scar of unshaped fibrous connective tissue with very thin epithelial cover is seen in their place. But patients with BD, as comparing to the control group, have lymphoid-plasmocytic microinfiltrations around small vessels in periaphthous sites even during remission period *(Figures 3, 4*).

Figure 3. Histological aspect of epithelia covering lamina propriae of oral mucous membrane during remission period of aphthous stomatitis (control group). Thickening of epithelium. Lamina propriae without features. Periaphthous zone. Patient C., 23 y.o. Hematoxylin-eosin, 480 x

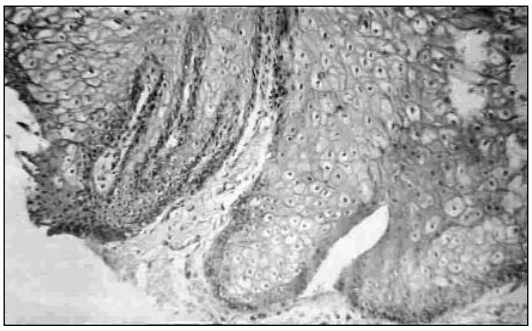
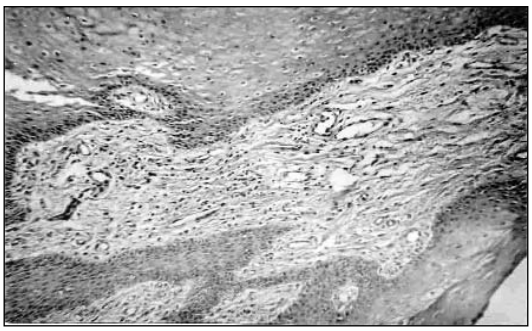


Figure 4. Histological condition of oral mucous membrane in patient with BD during remission period. Abundance of small, enlarged vessels of lamina propriae. Perivascular lymphoid-plasmocytic microinfiltrations. Periaphthous zone. Patient K., 27 y.o. Hematoxylin-eosin, 300 x

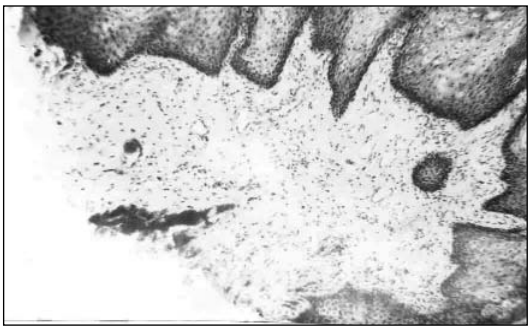


Mast cells

During aggravation period in patients of the control group, mast cells are noticed in lamina propriae of oral mucosa but not in epithelium. They possess strong metachromasia, are located individually, near small vessels (*Figure 5*).

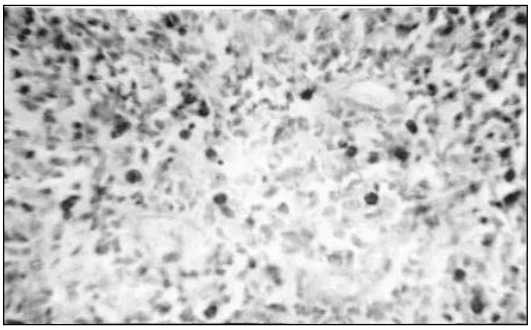
Their density of distribution is 7.9±1.1/mm^2, and percentage of degranulating mast cells reaches 4.0±0.2 % of their total population. Aphthaes zones, periaphthous and remote sites do not differ from one another in density of distribution and intensity of degranulation of these cells.

Figure 5. Microscopic condition of oral mucous membrane during aggravation, in patient with chronic recurrent aphthous stomatitis (control group). Small mast cells distributed rather regularly. They are absent outside perivascular congestions. Periaphthous zone. Patient F., 22 y.o. Buffer solution of thionine according to I.A Hassanov's method, 250 x



During aggravation period in patients of the main group (with BD) similarly to those of the control group, mast cells are detected only in lamina propriae of oral mucosa but not in epithelium and underlying muscle. In contrast to the reference group, these cells are localized in the inflammatory infiltrations as well as along the line of small vessels (*Figure 6*).

Figure 6. Microscopic condition of lamina propriae of oral mucous membrane during aggravation, in patient with BD. Abundance of chaotically scattered mast cells with primary perivascular localization. Periaphthous zone. Patient H., 30 y.o. Buffer solution of thionine according to I.A Hassanov's method + PAS-reaction, 480 x



Their maximal density is detected just in perivascular lymphoid-plasmocytic infiltrations. The average index of density of distribution is 17.4±2.0/mm^2 in aphthae zone;

12.2±1.4/mm^2 - in periaphthous sites and 6.0±0.8/mm^2 in remote sites.

Thus, the density gradient decreases from aphthae towards remote sites, which is not characteristic for recurrent aphthous stomatitis. The percentage of degranulation cells varies from 8-30 % of the population in the following way: 26.6-3.0 % aphthae; 18.2-2.1% - periaphthous sites; 10-1.8 % - remote sites. The emergence of bioactive substrates of mast cells is maximum just in the aphthae zone during the aggravation period of the disease. The discrete nature of distribution of degranulating cells is not characteristic for the patients of the control group.

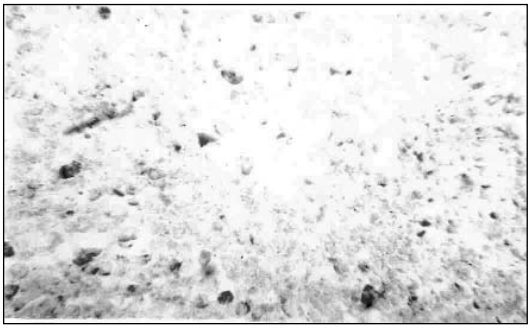
During remission period, the topography of mast cells in the patients with chronic recurrent aphthous stomatitis remains the same as during aggravation (*Figure 7*).

Figure 7. Histological condition of oral mucous membrane during remission, in patient with chronic recurrent aphthous stomatitis. Individual small lymphoid infiltrations in lamina propriae. Almost complete absence of mast cells. Periaphthous zone. Patient Y., 20 y.o. Buffer solution of thionine according to I.A Hassanov's method, 280 x

Density of distribution is without authentic changes: 7.0±1.3/mm^2 ($p>0.1$ as compared to aggravation period in the same group). The same situation is also characteristic for the percentage of degranulating cells: 3.8±0.8% ($p> 0.1$ as compared to aggravation period in the same group). No marked difference among quantitative indices over individual zones is revealed; in other words, there is no discreteness in the direction from aphtae towards remote sites.

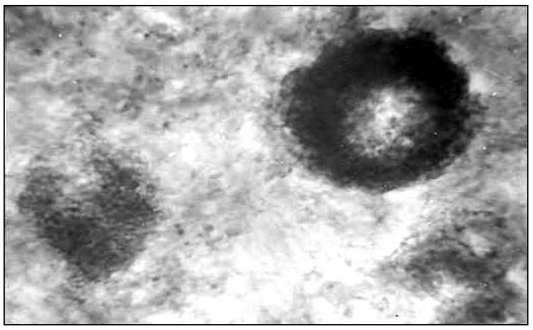
During remission periods of BD, MC are spread chaotically over lamina propriae of oral mucosa, they are predominantly located under the epithelium. The density of distribution of these cells is discrete, similarly to aggravation period; corresponding figures are not markedly different as compared to aggravation period of the process in the same patients (16.0±2.0/mm^2 in aphthae zone; 10.9±1.5/mm^2 - in periaphthous sites and 5.7±0.8/mm^2 in remote sites). But the percentage of actively degranulating mast cells in remission period of BD is authentically below. At the same time these indices are approximately similar over all the studied zones (aphthaes, periaphthous and remote sites): 5.5±0.9% (*Figure 8*).

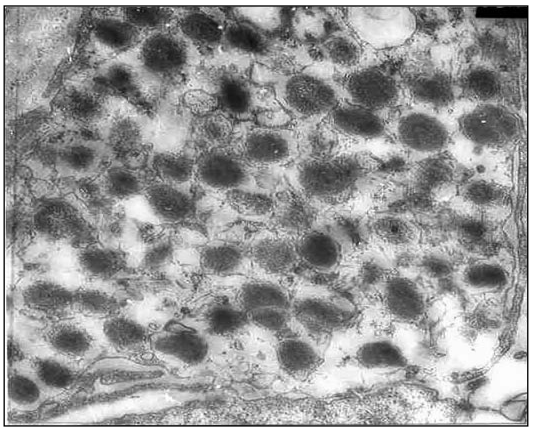
Figure 8. Histological condition of lamina propriae of oral mucous membrane during remission period in patient with BD. Almost the same quantity of mast cells with metachromatically reaction, but passive degranulating. Periaphthous zone. Patient H., 30 y.o. Buffer solution of thionine according to I.A Hassanov's method+ PAS-reaction, 480 x



So, transition of the process to an active phase in BD does not lead to an increase in density of distribution of mast cells in oral mucosa, but correlates with an enhancement of emergence of bioactive substratum. Strong and direct correlation between the frequency of aggravation and the percentage of actively degranulating cells ($r = 0.66$; $\chi^2 = 126.0$) is found for this index (*Figures 9,10*).

Figures 9, 10. Actively degranulating mast cells. Intensive emission of histamine. Lamina propriae of oral mucosa. BD, aggravation period. Patient R., 28 y.o. Fig. 9 - Buffer solution of thionine according to I.A Hassanov's method. Magnification: Fig 9: 1500 x (immersion); Fig. 10: 15000 x





Acid Glycosaminoglycans (GAG)

During remission periods of chronic recurrent aphthous stomatitis (control) and BD, the ratio of sulphated and non-sulphated acid GAG is 1.7/1.3. During aggravation of the process, histochemical contents of acid GAG increase authentically to maximum (3.8±0.4 mark) in BD but not in the patients of the control group. During the same period, the ratio of sulphated and non-sulphated acid GAG changes towards non-sulphated ones: 1.1/1.9 in the BD patients, as distinct from those of the control group.

The results of the present investigations let us confirm that aggravation of the disease is to be followed by the increase of the acid GAG content in the areas of aphthaes and periaphthouse zones. The kind of structural damages means that the microinfiltrates all around the fine vessels and in places of damaged area are the result of the anti-reaction of the body to its own cells. The investigations of these symptoms are very important to differentiate the BD from XRAS, to prevent the BD. The structural damages are less than in BD. This data can be used already in the future investigations of this difficult disease.

Conclusions

1. Aggravation of BD is accompanied by activation of mast cells. Transition of the process to an active phase in BD does not lead to an increase in density of distribution of mast cells in oral mucosa but directly correlates to a rise in emergence of bioactive substratum.
2. Aggravation of BD is accompanied by an enhancement of histochemical contents of acid GAG in aphthae zones and periaphthous sites of oral mucosa.
3. During aggravation of BD and formation of new aphthae, the ratio of sulphated and non-sulphated acid GAG authentically changes towards non-sulphated ones.
4. The condition of mast cells and acid GAG in oral mucosa may be of prognostic importance during aggravation of aphthous stomatitis of Behcet genesis.

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